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Prediction of the gas chromatographic relative retention times of flavonoids from molecular structure

Patricia Payares^{a,*}, Damaris Díaz^a, Jesús Olivero^b, Ricardo Vivas^b, Ismael Gómez^c

^aGroup of Theoretical Chemistry, Universidad de Cartagena, A.A. 6541, Cartagena, Colombia

^bDepartment of Chemistry, Universidad de Cartagena, Campus de Zaragocilla, Cartagena, Colombia

^cChemistry Department, Campus Cartuja, s/n, Universidad de Granada, A.A. 18071, Granada, Spain

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Abstract

Quantitative structure–retention relationships have been formulated for the gas chromatographic behaviour of 49 flavonoids in an apolar column, including flavones, flavonols, flavanones and a chalcone. Topological, geometric and electronic descriptors were obtained for model generation. Relationships between descriptors and the inverse form of the relative retention times of flavonoids relative to hispidulin were established using linear multiple regression. The best descriptors included in the model were $1/(\chi_C - \chi_C^V)$, and the sum of the values of the charges for the hydroxyl hydrogens. The predictive model had a correlation coefficient of 0.975 and a standard error of estimation of 0.120.

Keywords: Retention times; Retention prediction; Quantitative structure–retention relationships; Molecular descriptors; Flavonoids

1. Introduction

Flavonoids are natural products which occur in many families of plants [1]. Currently, flavonoids occupy an important place in natural products research. Their biological activity is extensive, and they have been used in traditional medicine as antiinflammatory [2], antimicrobial [3] and antifungal drugs [4]. In addition antimutagenic and anticancer activities have been reported [5,6]. Analyses of flavonoids are complicated due to their polarity, low solubility, similar functional groups and skeleton. Nevertheless, they have been analysed by gas [7,8] and liquid chromatography [9]. The structural identification of these compounds is made by GC–MS.

Therefore, sometimes the mass spectra does not give enough evidence for structure elucidation and a theoretical prediction model should be used to verify the molecular structure. This approach called quantitative structure retention relationships (QSRRs) generates useful equations for the prediction of relative retention times (RRTs) for molecules which are similar to those used to develop the model, but different from those included in the training set [10].

Many QSRR studies [11–15] have shown that the chromatographic behaviour can be predicted from molecular structure, giving information about the different molecular properties that may participate in the interactions between molecules and the stationary phase in GC.

The purpose of this paper is to present a statistical model that may be used to predict in a satisfactory

*Corresponding author.

way relative retention times for flavonoids. Also, the different chemical properties that may be contributing to the chromatographic retention of flavonoids in an apolar column are discussed.

2. Experimental

2.1. Data set

The data used in this research were reported by Schmidt et al. [7]. Relative retention times were determined for 49 flavonoids using a Hewlett-Packard HP 5890 gas chromatograph equipped with a Permabond OV-1 capillary column (25 m×0.25 mm I.D) with the following conditions: column temperature 300°C, nitrogen carrier gas at a flow-rate of 1.3 ml/min, split-ratio 1:50. RRTs were expressed relative to hispidulin and obtained after subtraction of the dead time (0.94 min).

2.2. Descriptor generation

Different parameters were generated for all the compounds in the data set. These numbers, commonly named molecular descriptors, were topological, geometric and electronic in nature. Topological descriptors describe molecular size and shape, factors which are important in chromatographic retention behaviour [11]. Topological descriptors include fragment and molecular connectivity indices. Fragment descriptors account for the number of substructures in the molecule, they include molecular mass and the number of atoms and bonds in the molecule. Calculated connectivity indices were the Randic [16], and Kier and Hall indices [17] and the content of information [18]. Spatial distribution of the atomic groups in the molecules is encoded by geometric descriptors such as the Van der Waals volume and surface [19], and the moments of inertia, which are widely used.

Two kinds of electronic descriptors were calculated: global and local descriptors. Global descriptors are related to the properties of the molecule as a whole. Local descriptors vary from point to point in space and can be obtained in an approximate form by integration over atomic regions or atoms in a molecule. The calculated global descriptors were the total

dipole moment, HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energies obtained by using the AM1 method [20], and also the molecular polarizability obtained by using the semiempirical method proposed by Miller and Savchik [21]. Atomic charges calculated via Gasteiger and Marsili's method [22] are one of the best known examples of local descriptors. In this work, atomic charges were calculated using the AM1 method and Gasteiger and Marsili's method in order to compare their importance on the prediction models. In this study, the obtained charge-derived descriptors were the sum of the absolute values of the charges in a given molecule (QT) accounting for charge separation in the molecule. The sum of values of the charges for the effective carbons (QECH), accounting for nonpolar regions. The sum of the charges for the heteroatoms (QHA), the most positive (MPC) and negative charges (MNC), related to local reactivity in the molecule.

A typical flavonoid showing the different atoms whose charges were generated appears in Fig. 1.

2.3. Computational details

Topological, geometric and electronic descriptors according to Gasteiger and Marsili's method were calculated with a 486/66 MHz personal computer, using PCDM, a program written in Turbo Pascal by our research group [23]. AM1 calculations were performed using the Gaussian program [24], running on a Digital-Alpha Station 200/166 MHz. Single point data were obtained from full AM1 optimized geometries.

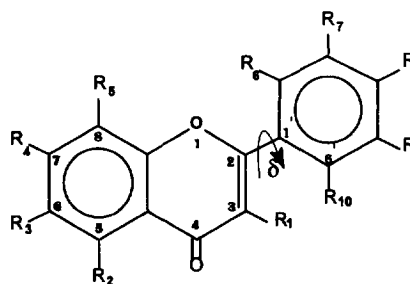


Fig. 1. Flavonoid molecule.

2.4. Statistical analysis

In order to reduce the number of descriptors prior to submitting them to regression routines, the concept of nonredundant descriptors NRD [25] was applied. When two descriptors are correlated by a linear correlation coefficient value higher than 0.9, both are correlated with the dependent variable. The one which presents the best correlation is used for further analysis, leaving out the descriptor showing lower correlation. Relationships between experimental RRTs and molecular descriptors were established following the multilinear form: $RRT = a_1D_1 + a_2D_2 + \dots + a_nD_n$, where D_1 , D_2 and D_n are the descriptors and a_1 , a_2 and a_n the respective regression coefficients. The final prediction equation was obtained using stepwise multiple regression techniques [26,27], applying the forward entry method. The best models were selected on the basis of the multiple correlation coefficient (R), standard error of estimation (S.E.), and F ratio value. The inclusion of the descriptors in the model was statistically justified when an F value of 4 was removed from the total variability.

2.5. Model validation techniques

The statistical validity of the best prediction model was tested using internal jack-knifing techniques [27]. This methodology was conducted leaving out one compound from the data set and regenerating the model coefficients. The correlation of these data with those obtained from the principal model yields the R_{crossval} [25]. Higher R_{crossval} values indicate whether the obtained model can be used to test flavonoid compounds which are different from those included in the data set.

3. Results and discussion

Molecular descriptors and regression coefficients of the best equation for the prediction of the chromatographic retention behaviour of flavonoids is given in Table 1. As shown, the best model was obtained when the inverse form of the dependent variable ($1/RRT$) was used. Fig. 2 is a plot of the predicted versus experimental $1/RRT$ values. Calculated

Table 1
Prediction model^a (OV-1 Column) for $1/RRT$ of flavonoids

| Descriptor | Regression coefficient \pm standard error |
|---------------------------|---|
| $1/(\chi_C^3 - \chi_C^Y)$ | 3.309 ± 0.484 |
| $(QECH)^2$ | 18.806 ± 2.632 |
| $1/E_{\text{LUMO}}$ | -0.786 ± 0.094 |
| SQHOH | 0.578 ± 0.125 |
| $1/Q6$ | 0.015 ± 0.005 |
| Intercept | -3.272 ± 0.462 |
| Statistical parameters | |
| R | 0.975 |
| SE | 0.120 |
| F | 183.588 |
| n | 49 |

^a $1/RRT = aD_1 + bD_2 + \dots + cD_n$. RRT: Retention times relative to hispidulin. a , b , c : Regression coefficients. D_1 , D_2 , D_n : Molecular descriptors.

lated descriptors for each one of the molecules used in this study are listed in Table 2. The R and S.E. values for the regression model were 0.975 and 0.120, respectively. According to analysis of variance, statistical significance was observed for the obtained model ($p < 0.001$).

Examination of the descriptors included in the model shows that the first one, $1/(\chi_C^3 - \chi_C^Y)$, is topological in nature. The square form of the sum of the values of the charges for the effective carbons $(QECH)^2$, the inverse form of the LUMO energy $(1/E_{\text{LUMO}})$, the sum of atomic charges for all the hydroxyl hydrogens (SQHOH), and the inverse form of the charge of the atom 6 ($1/Q6$) are electronic in nature. Among the electronic descriptors, $(QECH)^2$, SQHOH and $1/Q6$, which were calculated both via AM1 level and using Gasteiger and Marsili's method, only the $(QECH)^2$ values coming from the latter method gave better results in the prediction model.

The use of $1/RRT$ in the regression equation improved the predictive power of the model from 0.868 in the R value for the linear model to 0.975 in the same parameter for the inverse model. The correlation matrix (Table 3) for the descriptors included in the model shows that they are nonredundant. A cumulative correlation for all the descriptors is presented in Fig. 3, which shows that the first two descriptors account for the higher percent of the whole correlation.

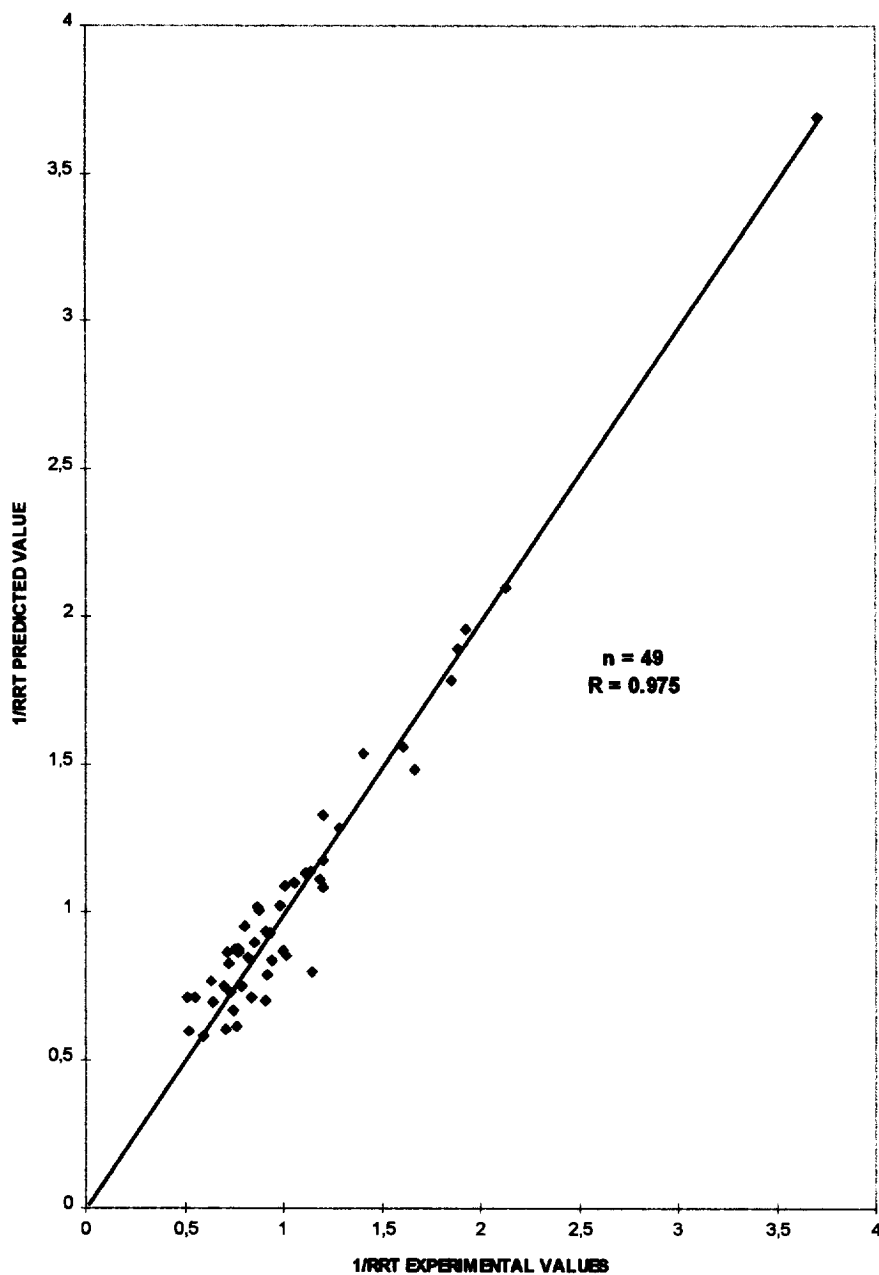


Fig. 2. Experimental versus predicted gas chromatographic 1/RRT for 49 flavonoids using the prediction model.

The descriptor $1/({}^3\chi_C - {}^3\chi_C^V)$ was the most significant in the model. ${}^3\chi_C$ encodes information about the number of branch points in a molecule. When calculating ${}^3\chi_C$ and ${}^3\chi_C^V$, δ and δ^V values were used, respectively, which means that both non-hydrogen

bonding sigma electrons, and π and lone pair electrons contribute to this descriptor. As a result, it can be inferred that this descriptor depends on the electronic distribution in the branched points of the molecules, which may interact unspecifically with

Table 2
Experimental $1/RRT$ for flavonoids in an OV-1 column and descriptors used in the QSRR model

| No. | Compounds | Experimental values (1/RRT) | $1/(^3\chi_C - ^3\chi_C^y)$ | $(QECH)^2 (\times 10^{-2})$ | $1/E_{LUMO}$ | SQHOH | 1/Q6 |
|-----|----------------------------------|-----------------------------|-----------------------------|-----------------------------|--------------|-------|---------|
| 1 | Apigenin | 0.980 | 0.890 | 0.117 | -1.120 | 0.845 | -2.834 |
| 2 | Genkwanin | 1.111 | 0.957 | 0.113 | -1.180 | 0.575 | -2.935 |
| 3 | Acacetin | 1.136 | 0.957 | 0.113 | -1.183 | 0.581 | -2.832 |
| 4 | Apigenin 7,4'-di-Me | 1.282 | 1.033 | 0.109 | -1.249 | 0.312 | -2.934 |
| 5 | Chrysoeriol | 0.820 | 0.841 | 0.115 | -1.104 | 0.845 | -2.833 |
| 6 | Diosmetin | 0.714 | 0.841 | 0.115 | -1.115 | 0.860 | -2.833 |
| 7 | Velutin | 0.935 | 0.901 | 0.119 | -1.160 | 0.575 | -2.935 |
| 8 | Piloin | 0.806 | 0.901 | 0.119 | -1.172 | 0.590 | -2.935 |
| 9 | Luteolin 7,3',4'-tri-Me | 0.877 | 0.955 | 0.121 | -1.218 | 0.312 | -2.934 |
| 10 | Hispidulin | 1.000 | 0.884 | 0.094 | -1.078 | 0.864 | -9.962 |
| 11 | Pectolinarigenin | 1.149 | 0.950 | 0.091 | -1.135 | 0.287 | -9.928 |
| 12 | Cirsimaritin | 0.758 | 0.936 | 0.092 | -1.129 | 0.575 | -12.665 |
| 13 | Salvigenin | 0.869 | 1.011 | 0.089 | -1.191 | 0.312 | -12.627 |
| 14 | Jaceosidin | 0.840 | 0.837 | 0.140 | -1.063 | 0.864 | -9.944 |
| 15 | Desmethoxycentaureidin | 0.730 | 0.837 | 0.140 | -1.073 | 0.880 | -9.937 |
| 16 | Eupatilin | 0.787 | 0.883 | 0.142 | -1.111 | 0.600 | -9.909 |
| 17 | Cirsilineol | 0.645 | 0.883 | 0.142 | -1.112 | 0.575 | -12.649 |
| 18 | Eupatorin | 0.549 | 0.883 | 0.142 | -1.123 | 0.591 | -12.647 |
| 19 | Tricin | 0.943 | 0.792 | 0.920 | -1.091 | 0.864 | -2.835 |
| 20 | Apigenin 6,3',5'-tri-OMe | 0.513 | 0.788 | 0.988 | -1.051 | 0.884 | -9.958 |
| 21 | Nevadensin | 0.775 | 0.926 | 0.112 | -1.111 | 0.601 | -11.184 |
| 22 | Galangin | 1.923 | 1.012 | 3.327 | -1.009 | 0.867 | -2.804 |
| 23 | Kaempferol | 0.909 | 0.832 | 0.253 | -1.014 | 1.128 | -2.806 |
| 24 | Kaempferid | 1.010 | 0.890 | 0.247 | -1.182 | 0.839 | -2.814 |
| 25 | Kaempferol 3,7-di-Me | 1.205 | 0.932 | 0.247 | -1.189 | 0.573 | -2.926 |
| 26 | Kaempferol 3,4'-di-Me | 1.053 | 0.932 | 0.338 | -1.181 | 0.581 | -2.825 |
| 27 | Kaempferol 7,4'-di-Me | 1.190 | 0.956 | 0.241 | -1.114 | 0.594 | -2.903 |
| 28 | Kaempferol 3,7,4'-tri-Me | 1.205 | 1.005 | 0.241 | -1.198 | 0.311 | -2.922 |
| 29 | Isorhamnetin | 0.752 | 0.789 | 0.032 | -1.120 | 0.835 | -2.813 |
| 30 | Tamarixetin | 0.637 | 0.789 | 0.032 | -1.016 | 1.143 | -2.804 |
| 31 | Dillenetin | 0.699 | 0.831 | 0.033 | -1.025 | 0.866 | -2.805 |
| 32 | Quercetin 3,7,3'-tri-Me | 0.909 | 0.880 | 0.034 | -1.170 | 0.311 | -2.925 |
| 33 | Quercetin 3,7,4'-tri-Me | 0.769 | 0.880 | 0.034 | -1.185 | 0.588 | -2.925 |
| 34 | Quercetin 3,7,3',4'-tetra-Me | 0.855 | 0.931 | 0.035 | -1.204 | 0.311 | -2.926 |
| 35 | 6-Methoxykaempferol | 0.917 | 0.827 | 0.219 | -0.973 | 1.147 | -9.675 |
| 36 | Betuletol | 1.020 | 0.883 | 0.213 | -1.008 | 0.885 | -9.648 |
| 37 | Penduletin | 0.725 | 0.913 | 0.216 | -1.134 | 0.573 | -12.508 |
| 38 | Spinacetin | 0.763 | 0.785 | 0.045 | -0.966 | 1.147 | -9.654 |
| 39 | Quercetagenin 6,3',4'-tri-Me | 0.704 | 0.826 | 0.047 | -0.973 | 0.886 | -9.675 |
| 40 | Veronicafolin | 0.592 | 0.826 | 0.046 | -1.012 | 0.856 | -12.112 |
| 41 | Quercetagenin 3,6,7,4'-tetra-Me | 0.521 | 0.862 | 0.047 | -1.088 | 0.589 | -12.476 |
| 42 | Naringenin | 1.667 | 0.932 | 0.276 | -1.690 | 0.576 | -2.755 |
| 43 | Sakuranetin | 1.887 | 1.005 | 0.269 | -1.912 | 0.567 | -2.852 |
| 44 | Isosakuranetin | 1.852 | 1.005 | 0.269 | -1.765 | 0.576 | -2.753 |
| 45 | Naringenin 7,4'-di-Me | 2.128 | 1.091 | 0.263 | -2.006 | 0.307 | -2.850 |
| 46 | Hesperetin | 1.205 | 0.879 | 0.025 | -1.582 | 0.838 | -2.760 |
| 47 | Eriodictyol 7,3'-di-Me | 1.613 | 0.943 | 0.027 | -1.787 | 0.587 | -2.855 |
| 48 | Persicogenin | 1.408 | 0.943 | 0.027 | -1.778 | 0.569 | -2.857 |
| 49 | 2,6-Dihydroxy-4'-methoxychalcone | 3.704 | 1.247 | 6.891 | -1.629 | 0.536 | -3.596 |

Table 3
Correlation matrix for the descriptors used in the prediction model

| | $1/(\chi_C^{-3}\chi_C^V)$ | $(QECH)^2$ | $1/E_{LUMO}$ | SQHOH | 1/Q6 |
|---------------------------|---------------------------|------------|--------------|--------|-------|
| $1/(\chi_C^{-3}\chi_C^V)$ | 1.000 | | | | |
| $(QECH)^2$ | 0.569 | 1.000 | | | |
| $1/E_{LUMO}$ | -0.604 | -0.157 | 1.000 | | |
| SQHOH | -0.662 | -0.004 | 0.427 | 1.000 | |
| 1/Q6 | 0.240 | 0.126 | -0.396 | -0.098 | 1.000 |

Number of data=49. The internal standard is included.

the stationary phase. Furthermore, it has been suggested that $\delta-\delta^V$ is in some grade a function of electronegativity [17].

SQHOH indicates the charges of hydroxyl groups present in the molecule. This descriptor may be associated with the formation of intra and inter-molecular hydrogen bonds [9].

$1/E_{LUMO}$ has been related to the molecular capability of accepting electrons, and this descriptor may be associated with the formation of charge transfer complexes between molecules and the stationary phase [28]. The higher values of LUMO energy, the higher molecular capacity of receiving electrons. The theoretical aspects of the charge transfer complexes [29] assume the existence of the complex is due to interactions between the HOMO of the donor with the LUMO of the acceptor. In this context, being the stationary phase of apolar nature (methyl groups), the idea of electronic transfer from these groups may not be well explained. In this case, the existence of a different mechanism should be considered. From the complete correlation matrix for all the descriptors, the descriptor which best correlates with E_{LUMO} was the torsional angle δ (Fig. 1 atoms 3, 2, 1' and 6') with an R value of -0.802 . However, for descriptor

selection, when E_{LUMO} and the torsional angle δ were correlated against RRT, the best result was obtained with the E_{LUMO} ($R=-0.67$ vs. $R=0.52$). In short, It is quite reasonable to suggest that the flavonoid planarity may play a role in the ability of these compounds to interact electronically with the stationary phase.

The inverse form of the charge for atom 6, namely 1/Q6, depends on the different substitutions in ring A for a flavonoid. When a molecule is substituted with a methoxy group in position 6, the RRT value is increased. The QSRR model evidently shows a preference for 1/Q6, rather than Q5. This may be explained considering that the Q6 environment is affected by both Q6 and Q8 substituents common in flavonoids.

The sum of values of the charges for effective carbons, namely QECH, is an electronic descriptor which encodes information about the relative position of groups in the whole molecule. Calculating this descriptor using Gasteiger and Marsili's method was much more effective than the AM1 method during the selection of the descriptors in the generation model process.

Factor analysis was used to detect intercorrelations between selected variables in the best model. This statistical procedure consists of condensing the information encoded in a group of original variables (molecular descriptors) in a new reduced group of variables named factors, where each factor is a linear combination of the original variables. In this work, all those factors which account for more than 95% of the cumulative percent of the total variability were included in the analysis. Four factors were extracted from the descriptors involved in the model. The first factor explains 48.6% of the total variability, the second 20.7%, the third 19.4% and the fourth 9.2% for a total of 98.0%. In order to obtain a better

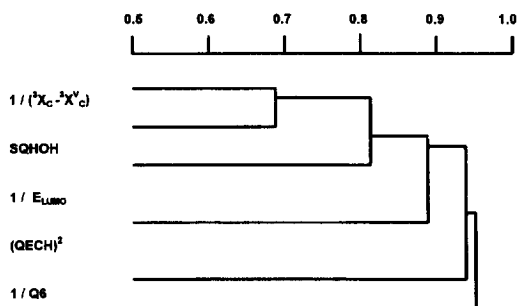


Fig. 3. Cumulative correlation for all the descriptors included in the prediction model.

Table 4
Varimax rotated factor matrix for descriptors in the OV-1 model

| Variable | Factor | | | |
|---------------------------|---------------------|--------|--------|--------|
| | 1 | 2 | 3 | 4 |
| $1/(\chi_C^3 - \chi_C^V)$ | -0.654 ^a | 0.583 | -0.406 | 0.101 |
| $(QECH)^2$ | -0.002 | 0.988 | -0.048 | 0.054 |
| $1/E_{LUMO}$ | 0.253 | -0.092 | 0.937 | -0.213 |
| SQHOH | 0.972 | 0.029 | 0.180 | -0.027 |
| $1/Q6$ | -0.042 | 0.065 | -0.183 | 0.980 |

^a Factorial coefficients of the variables: correlation between the descriptors and the factors.

interpretation from our factor analysis, the extracted principal factors were rotated applying the Varimax method (Table 4). It can be seen that the first factor is directly related to SQHOH and inversely related to $1/(\chi_C^3 - \chi_C^V)$, the second is associated with $(QECH)^2$, the third with $1/E_{LUMO}$ and the fourth with $1/Q6$.

The internal jack-knifing validation for the OV-1 model gave a $R_{crossval}$ value of 0.978, meaning that the model is able to predict relative retention times.

4. Conclusions

Results of this study demonstrate that the QSRR can generate a very good model for the relative retention times of flavonoids in an apolar column, when complementary electronic and topological descriptors are included. The descriptors included in the prediction model give information related to the different molecular properties which can participate in the physicochemical process in gas chromatographic retention of flavonoids.

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